

ASEXUAL REPRODUCTION IN THE COLONIAL TUNICATE,
BOTRYLLUS SCHLOSSERI (PALLAS) SAVIGNY, WITH
SPECIAL REFERENCE TO THE DEVELOPMENTAL
HISTORY OF INTERSIPHONAL BANDS OF
PIGMENT CELLS

RAY L. WATTERSON¹

Marine Biological Laboratory and University of California, Berkeley

INTRODUCTION

Botryllus schlosseri (Pallas) Savigny² is a colonial tunicate readily obtained in the Eel Pond at Woods Hole, Massachusetts. In a well developed colony the individual blastozooids (or ascidiozooids) are grouped into one or more systems; each system consists of 2 to 23 blastozooids radiating outwards from a central common cloaca with the separate oral siphons distributed at the periphery. The most striking feature of many *Botryllus* colonies is the localization of special light-reflecting pigment cells between the oral and atrial siphons of each blastozooid. Because of the association of individual blastozooids into systems within each colony the total aggregation of such reflecting cells within any one system forms an attractive star-shaped pattern (Pl. 1, Fig. 3) clearly revealing the spatial distribution of the blastozooids involved. Such patterns will be referred to as intersiphonal patterns of pigment cells (intersiphonal patterns for short), and each arm of the pattern will be called an intersiphonal band of pigment cells (intersiphonal band for short).

Such intersiphonal bands have been described more or less incidentally by various taxonomists; however, only two of these seemed to realize that the bands are actually aggregations of pigment cells in specific regions. Both of these men realized that these intersiphonal bands are not constant in their appearance. Pizon (1899a) merely states that they undergo changes with time without specifying the nature of these changes. Bancroft (1903) states that in young zooids there are no intersiphonal bands at all and that three to four days elapse before they reach their complete formation. Such brief observations constitute the only information available in the literature concerning the developmental history of intersiphonal bands

¹ It is a pleasure to acknowledge the excellent assistance of Miss Juanita Senyard during the accumulation of the data upon which this paper is based.

² Many species and varieties of *Botryllus* have been described (Giard, 1872; Herdman, 1891; Hartmeyer, 1909-1911; Alder and Hancock, 1912). However, these were distinguished largely on the basis of color differences. Pizon (1899a) early recognized the need to revise the classification of *Botryllus* since he realized that considerable color variation may occur even within the same colony, and Bancroft (1903) even went so far as to state (p. 161). "in *Botryllus*, as it occurs in Europe and the Atlantic Coast of North America, color characters cannot be used for separating species; . . . therefore, since none of the described species have been based upon morphological characters, there is no valid reason for recognizing more than the single species, *B. schlosseri* (Pallas, 1766, pp. 355-356) Savigny (1816)." This view is acceptable to Van Name (1910) and to Herdman (1925).

of pigment cells. This lack of information is rather surprising since the bands are so very striking when fully formed (Pl. I, Fig. 5) and in view of the fact that so much has been written concerning other features of *Botryllus*.

One of the most interesting features of these intersiphonal bands has received no attention at all, viz., that they are not permanent additions to the pigment pattern of the colony; instead, as soon as they have formed, they are completely destroyed, and this destructive phase marks the most radical change in the appearance of the colony (Pl. II, Fig. 8). This destructive phase is inevitable since, as is well known (Berrill, 1941c), the parent zooids degenerate each time a new generation of zooids arises by asexual reproduction, and these intersiphonal bands are properties of the individual zooids, not of the colony as a whole. The fact that they give to the colony a characteristic intersiphonal pattern or group of patterns is only secondary.

This paper has a twofold purpose: (1) To describe the visible changes in the intersiphonal bands of pigment cells during the establishment of the colony from the larva. By observing the development of a number of colonies it is possible to correlate rather accurately the steps involved in the formation and destruction of the intersiphonal bands with other known steps in asexual reproduction. (2) To describe the variation in the intersiphonal patterns when different colonies are compared, and to consider possible factors involved in such variations.

MATERIALS AND METHODS

The eggs of *Botryllus* undergo fertilization and development up to a tadpole stage within the blastozooids. Each day some of these tadpoles escape from the cloaca. In order to obtain them adult colonies were collected from the Eel Pond in the morning and in the laboratory they were distributed in ten inch finger bowls filled with sea water. As the larvae escape from the parent zooids they swim toward the light and toward the surface (see Grave and Woodbridge, 1924, for time of liberation of larvae and for reactions of the larvae to light and gravity) and they can be collected easily with a pipette; three of them were placed into a large drop of sea water in the center of a syracuse watch glass and were left to attach and metamorphose into the oozoid. The watch glasses were stacked to prevent evaporation. Many of these tadpoles metamorphose without attaching (Grave, 1937) or exhibit certain indications of abnormal metamorphosis (Zhinkin, 1939), but these were discarded. Usually at least one tadpole attached in each watch glass; in some instances two or even all three larvae attached, and if they were sufficiently isolated from one another, they were allowed to continue their development. After attachment had occurred the watch glasses were immersed in large aquaria through which sea water was circulating. The watch glasses were inverted so debris would not settle out too thickly and obscure the development of the colonies, and they were held in this position by wooden racks. Each day these watch glasses were removed from the aquaria and the appearance of the colonies developing within them was sketched under the dissecting microscope with reflected light for illumination, since by such illumination only the distribution of the special light reflecting pigment cells is revealed and all other pigmentation of the zooids is automatically eliminated from observation (see Plates I and II). The exact time at which each sketch was made was recorded. Whenever a colony was

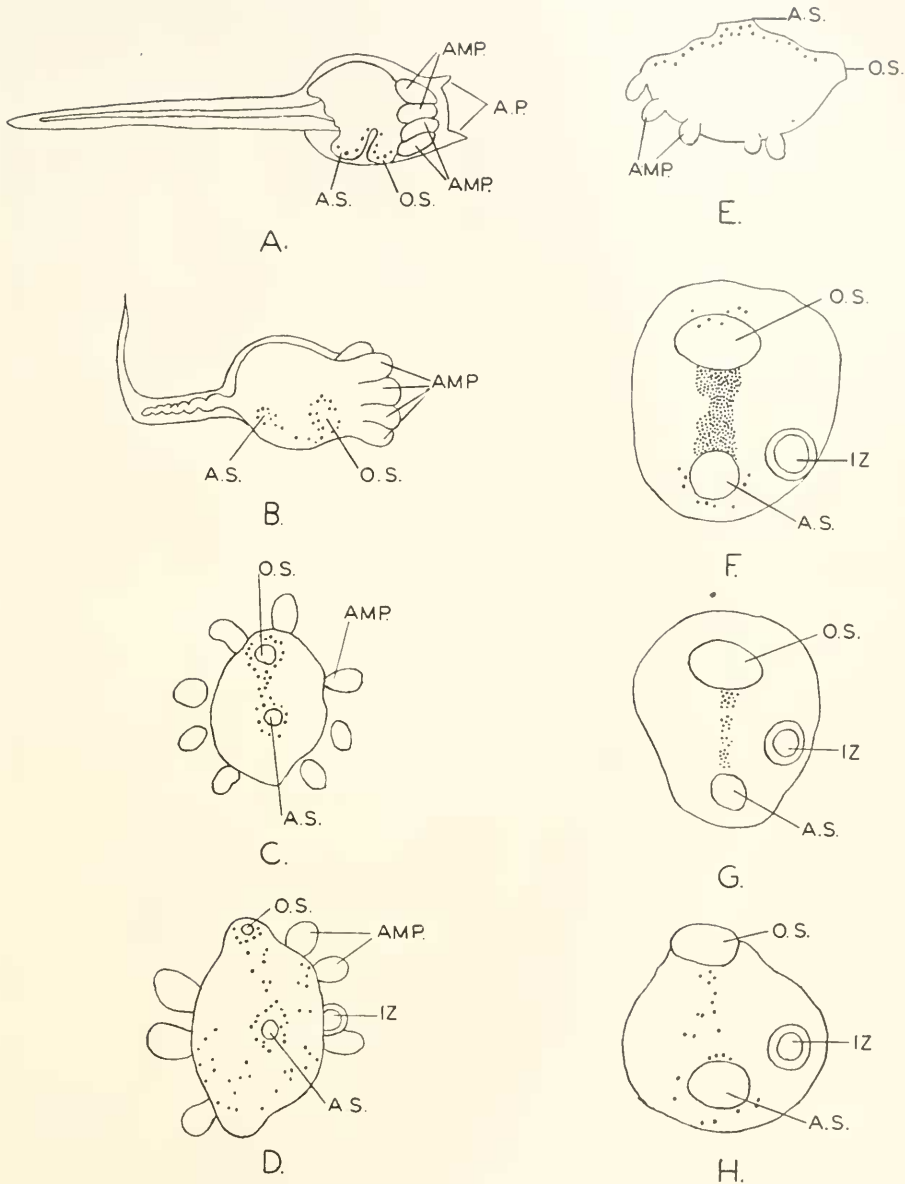


FIGURE 1. The development of intersiphonal bands of pigment cells in the oozoid. Each dot represents one pigment cell. a. Botryllus tadpole 20 to 30 minutes after escape from parent zooid. b. Tadpole in which the tail is undergoing absorption; one hour after escape from parent zooid. c. Oozoid eight hours after escape from parent zooid. d. Another oozoid 11 hours after escape from parent zooid. e. An optical section of an oozoid eight hours after escape from parent zooid. f. Oozoid No. 8c; age two days; example of a *strong* intersiphonal band of pigment cells. g. Oozoid No. 10; age two days; example of a *medium* intersiphonal band. h. Oozoid No. 18c; age two days; example of a *weak* intersiphonal band. Abbreviations: a.p. = adhesive papillae; amp. = ampullae; a.s. = atrial siphons; o.s. = oral siphons; IZ = first generation blastozooids.

in a crucial stage of transformation it could be watched for considerable periods of time without injury. However, because of the large number of colonies studied, continuous observations on any one colony for long periods of time were not possible, although such a procedure would have yielded useful supplementary information. All sketches made for each colony were then mounted from left to right in a horizontal row on large sheets of paper in the order in which the sketches were made, and they were so arranged on these sheets that sketches of all colonies made on any one day lie in vertical columns. Consequently the changes in the appearance of any one colony throughout the period studied can be ascertained quickly by running the eye horizontally across the sheet; and similarly the appearance of all the colonies on a given day is readily compared by running the eye vertically down the sheet. Sixty-one colonies were started from isolated larvae on July 31, 1942. These were first sketched on August 2, next on August 4, and then at daily intervals until August 19 if they still survived. Due to the increased complexity of the colonies by this time it was impossible to continue daily observations on all of them. Ten colonies were therefore selected and were sketched at daily intervals until September 4. Twenty-nine other colonies were allowed to develop until August 29 when sketches were made of them; however, for these colonies the daily changes between August 19 and August 29 are unknown. In the case of the remaining 22 colonies no sketches were made after August 19 for various reasons. Consequently, since only incomplete records are available for these 22 colonies, they will not be included in the general observations given below. In order to present these observations the daily changes in the appearance of one colony (No. 38) selected as an example will be described in detail (Fig. 2); the observations on the other colonies can then be presented briefly (Table I) by merely emphasizing their similarities and differences when compared with this example. All observations have been made exclusively on living material.

OBSERVATIONS

Formation and Variation of Intersiphonal Bands in Oozoids

Since the main series of colonies started on July 31 was first examined two days later it was necessary to start other colonies (on August 31 and September 3) in order to study the establishment of the first intersiphonal band during metamorphosis of the tadpole into the oozoid. These colonies served only for these early observations on oozoid development and were then discarded. Although the tadpole is rather opaque a few reflecting pigment cells can sometimes be seen in a newly emerged larva (Fig. 1a). By transmitted light these cells appear yellowish and by reflected light they are just barely visible. They are quite definitely localized in the dorsal surface and already outline the siphonal areas. Within an hour after the escape of the larva from the parent these pigment cells have darkened

FIGURE 2. The developmental history of intersiphonal bands of pigment cells in colony No. 38. Each dot represents a pigment cell. See text for age of colony at stages illustrated and for description of the progressive changes. The various stages are not drawn accurately to scale. Each degenerating zooid is indicated by cross-hatching. Abbreviations: c.c. = common cloaca; o.s. = oral siphons; s. = space free from pigment cells; 1Z to 7Z = first to seventh generation blastozooids.

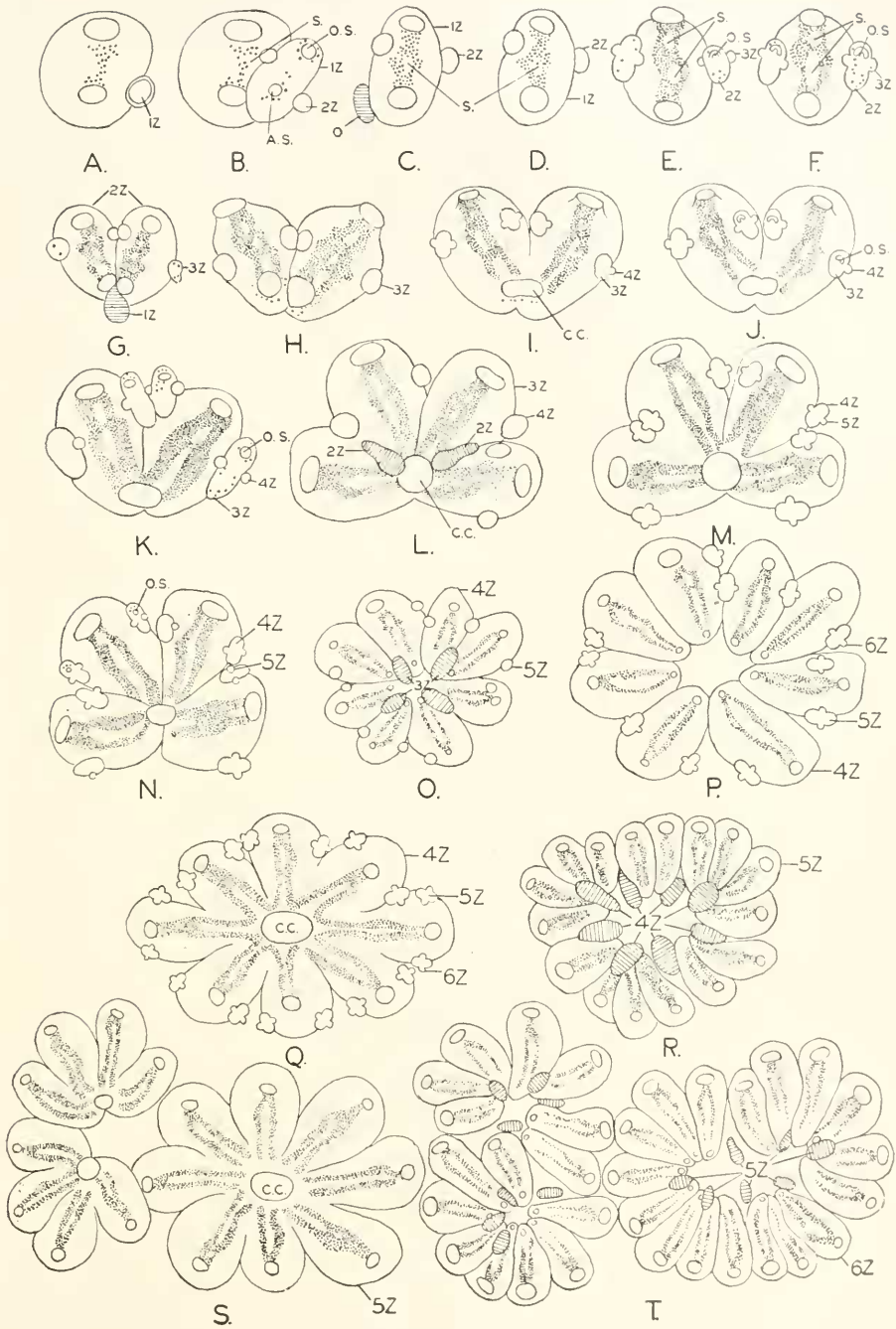
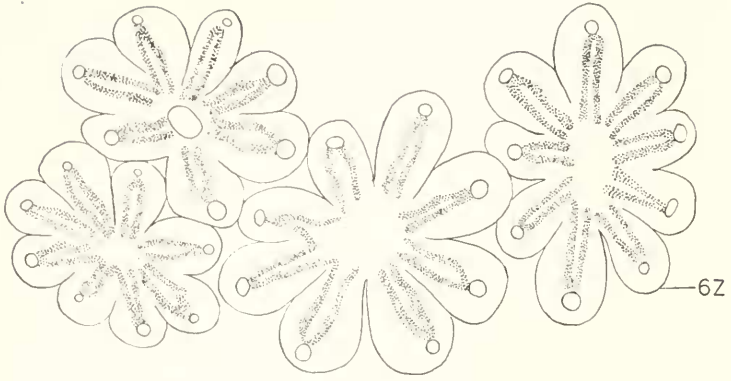
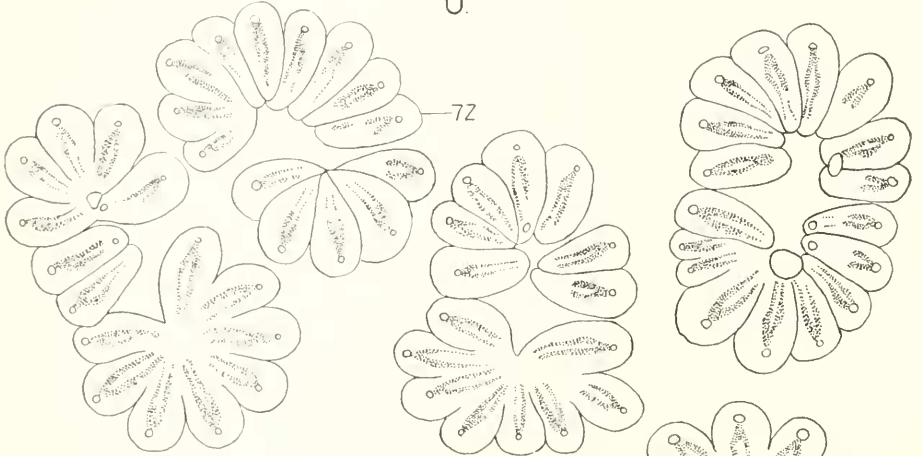


FIGURE 2



U.



V.



W.

FIGURE 2—Continued

TABLE I

Summary of the relationship between changes in the intersiphonal bands of pigment cells and the major changes in asexual reproduction in all colonies studied. Each number indicates the percentage of colonies undergoing the change indicated. The number in parentheses following each percentage indicates the generation of zooids involved (O = oozoid, 1-7 = first 7 generations of blastozooids). The percentages indicated by the asterisk may be too small since parent zooids obscure the buds in advanced stages of colony formation.

Age in days	Zooids degenerating	Pigment cells circulating	Inter-siphonal bands single	Inter-siphonal bands double	Buds enlarging	Oral siphons present	Atrial siphons present	Common cloacas formed	Non-growing buds present
5	59.0(0)	30.7	33.3(1)	0.0(1)	59.0(2)	0.0(2)	0.0(2)	0.0(2)	0.0(3)
6	41.0(0)	33.3	61.5(1)	5.1(1)	94.9(2)	0.0(2)	0.0(2)	0.0(2)	17.9(3)
7	2.6(0)	7.7	5.1(1)	56.4(1)	100.0(2)	15.4(2)	0.0(2)	0.0(2)	94.9(3)
8	0.0(0)	7.7	7.7(1)	56.4(1)	100.0(2)	51.3(2)	28.2(2)	0.0(2)	100.0(3)
9	35.9(1)	35.9	17.9(2)	2.6(2)	15.4(3)	94.9(2)	94.9(2)	0.0(2)	0.0(4)
10	41.0(1)	23.0	28.2(2)	25.6(2)	82.1(3)	100.0(2)	100.0(2)	23.0(2)	7.7(4)
11	7.7(1)	2.6	20.5(2)	43.6(2)	97.4(3)	100.0(2)	100.0(2)	66.7(2)	74.1(4)
12	2.6(1)	12.8	7.7(2)	56.4(2)	100.0(3)	23.0(3)	0.0(3)	69.2(2)	97.4(4)
13	23.0(2)	12.8	2.6(2)	51.3(2)	5.1(4)	41.0(3)	5.1(3)	0.0(3)	97.4(4)
14	48.7(2)	38.5	30.7(3)	2.6(3)	48.7(4)	41.0(3)	41.0(3)	7.7(3)	2.6(5)
15	23.0(2)	7.7	17.9(3)	28.2(3)	84.6(4)	84.6(3)	84.6(3)	51.3(3)	20.5(5)
16	5.1(2)	2.6	7.7(3)	48.7(3)	100.0(4)	2.6(4)	0.0(4)	66.7(3)	76.9(5)
17	5.1(3)	2.6	0.0(3)	56.4(3)	100.0(4)	25.6(4)	0.0(4)	0.0(4)	89.7(5)
18	23.0(3)	7.7	5.1(4)	5.1(4)	20.5(5)	56.4(4)	15.4(4)	5.1(4)	2.6(6)
19	23.0(3)	17.9	23.0(4)	17.9(4)	87.2(5)	94.9(4)	76.9(4)	38.5(4)	10.3(6)
20	0.0(3)	0.0	10.0(4)	80.0(4)	100.0(5)	100.0(4)	90.0(4)	90.0(4)	20.6(6)
21	10.0(3)	10.0	10.0(4)	80.0(4)	100.0(5)	100.0(4)	90.0(4)	90.0(4)	80.0(6)
22	10.0(4)	10.0	0.0(5)	10.0(5)	90.0(5)	10.0(5)	0.0(5)	0.0(5)	100.0(6)
23	40.0(4)	40.0	10.0(5)	50.0(5)	60.0(6)	60.0(5)	60.0(5)	50.0(5)	0.0(7)
24	20.0(4)	20.0	10.0(5)	80.0(5)	90.0(6)	90.0(5)	90.0(5)	80.0(5)	0.0(7)
25	0.0(4)	0.0	0.0(5)	90.0(5)	100.0(6)	90.0(5)	90.0(5)	90.0(5)	0.0(7)
26	10.0(4)	20.0	0.0(5)	100.0(5)	100.0(6)	100.0(5)	100.0(5)	90.0(5)	30.0(7)
27	10.0(5)	10.0	0.0(6)	10.0(6)	100.0(6)	10.0(6)	10.0(6)	0.0(6)	*30.0(7)
28	30.0(5)	10.0	0.0(6)	40.0(6)	100.0(6)	40.0(6)	20.0(6)	20.0(6)	*40.0(7)
29	10.3(5)	12.8	2.6(6)	35.9(6)	100.0(6)	61.5(6)	61.5(6)	51.3(6)	100.0(7)
30	0.0(5)	0.0	0.0(6)	100.0(6)	100.0(7)	100.0(6)	100.0(6)	100.0(6)	0.0(8)
31	0.0(6)	0.0	0.0(6)	100.0(6)	100.0(7)	100.0(6)	100.0(6)	100.0(6)	*0.0(8)
32	0.0(6)	0.0	0.0(7)	10.0(7)	100.0(7)	10.0(7)	10.0(7)	10.0(7)	*0.0(8)
33	20.0(6)	0.0	0.0(7)	40.0(7)	100.0(7)	40.0(7)	40.0(7)	10.0(7)	*0.0(8)
34	20.0(6)	20.0	0.0(7)	80.0(7)	100.0(7)	70.0(7)	60.0(7)	60.0(7)	*0.0(8)
35	0.0(6)	0.0	0.0(7)	100.0(7)	100.0(7)	100.0(7)	100.0(7)	100.0(7)	*0.0(8)

until they appear brownish by transmitted light and are more readily visible by reflected light; they now outline the siphonal region rather sharply (Fig. 1b). In some instances one or two of these reflecting cells are also visible in the ampullae (amp., Figs. 1a-1e). No great increase in the number of reflecting cells seems to take place during metamorphosis, at least not during the first day. Seven to eight hours after emergence of the larva the siphons are readily visible and typically the reflecting cells are restricted sharply to the areas immediately surrounding and between the siphons (Fig. 1c); however, in a few instances they are more widely distributed to either side of the dorsal midline as well (Fig. 1d). That these cells are very definitely restricted to the dorsal surface of the oozoid can be seen in optical section (Fig. 1e).

When the oozoids of the colonies started July 31 were first examined on August 2 considerable variation was noted in the appearance of their intersiphonal bands; however, the intersiphonal band of any oozoid could be classified quite readily as one of three types, either: a. *strong*, in which case there is a broad, almost solid band of reflecting cells between the two siphons (Fig. 1f); b. *medium*, in which case there is a band of pigment cells between the two siphons, but it is usually rather narrow or irregular and consists of relatively few reflecting cells (Fig. 1g); c. *weak*, in which case there are only a few scattered reflecting cells near the dorsal midline (Fig. 1h) with no obvious arrangement into a band. Twenty-nine of the colonies studied arose from oozoids classified as possessing *strong* intersiphonal bands, 13 from oozoids with *medium* intersiphonal bands and 16 from those with *weak* bands. Three colonies had abnormal oozoid stages in which the intersiphonal bands could not be classified.

Destruction of Intersiphonal Bands in Oozoids and Their Formation in First Generation Blastozoids

Each oozoid reproduces asexually by budding to produce the first generation blastozoid (1Z).³ This bud is a double walled structure located on the right side of the oozoid; its inner layer is an evagination of part of the atrial wall of the parent oozoid; its outer layer is an evagination of the overlying epidermis. The atrial portion of the bud consists of formative cells which give rise to all parts of the blastozoid except the epidermis which is derived directly from the epidermis of the parent. By the time the siphons of the first generation blastozoid are established the oozoid begins to degenerate; its intersiphonal band is destroyed and the oozoid itself almost completely disappears. Meanwhile an intersiphonal band is established in the first blastozoid.

³ The details of asexual reproduction in Botryllus have been presented rather fully elsewhere (Della Valle, 1882; Hjort, 1892; Oka, 1892; Pizon, 1893; Berrill, 1941a, 1941b, 1941c). However, some indication of the process is necessary here and also at other points in order to understand the progressive changes in the intersiphonal patterns recorded in this paper.

PLATE I

All figures are photographs of the dorsal surface of the colonies taken by reflected light. Magnification *ca.* 7×.

FIGURE 3. Colony 30c. Age 33 days. 6Z zooids with well developed intersiphonal bands.

FIGURE 4. Colony 35. Age 28 days. 6Z zooids distributed in two systems. Intersiphonal bands only partially developed and therefore not completely double. 5Z zooids degenerated on the 27th day.

FIGURE 5. Colony 23. Age 29 days. 6Z zooids distributed in four systems. Intersiphonal bands well developed.

FIGURE 6. Colony 13C. Age 28 days. Zooids highly sensitive and contractile resulting in the contracted state and irregularity of the intersiphonal patterns. Reflecting pigment cells are just appearing in the circulatory system. This is the first stage of the destruction of intersiphonal patterns. On the 29th day these 5Z zooids undergo degeneration and are replaced by 6Z zooids.

FIGURE 7. Colony 41. Age 28 days. 5Z zooids with well developed double intersiphonal bands. Nine 6Z zooids are faintly visible between the 5Z zooids. Note that intersiphonal bands are already appearing on 6Z zooids (arrow) before 5Z zooids have contracted. This is an exceptional condition.

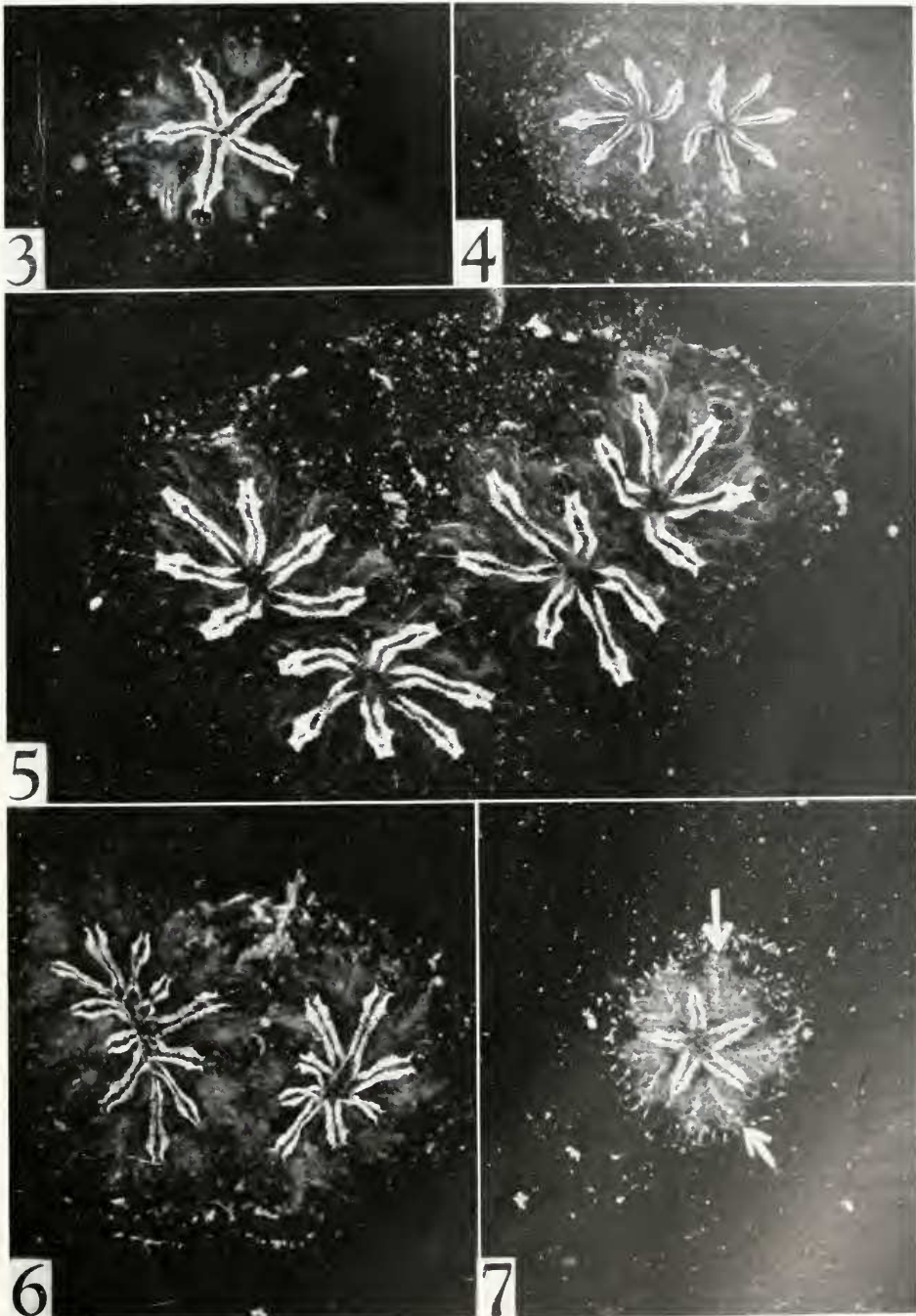


PLATE I

The asexual bud which will develop into the first generation blastozoid is already present in the tadpole before its emergence from the parent zooid (Pizon, 1893). It is readily visible on all oozoids the second day after the tadpole has attached (1Z in Figs. 1f, 1g, 1h and 2a). Sometime during the third day the bud begins to grow rapidly; a few scattered reflecting cells may then be present in the bud.

Fourth day (Fig. 2b). Both siphons are now visible in the first generation blastozoid. Reflecting cells are quite definitely restricted to the region immediately surrounding the siphons, with a few cells between the siphons and even to either side of the mid-dorsal line in some colonies. Meanwhile in the oozoid there is a tendency for the reflecting cells to become rearranged in part of the intersiphonal band, leaving spaces free from reflecting cells; such a space is forming in colony No. 38, although it is very small. In other oozoids the rearrangement of reflecting cells on the dorsal surface is much more extensive, especially near the oral siphon, serving to split that part of the originally single intersiphonal band into a double band. Occasionally reflecting cells may disappear first from other parts of the band. In three of the colonies the intersiphonal band appears somewhat contracted; in one colony some of the reflecting cells have been swept into the ampullae⁴ by way of the circulatory system. The buds of the second generation blastozoids (2Z) are already present. Typically one bud forms on the right side and one on the left of the first blastozoid (although sometimes only one bud forms, in which case it is always on the right side; in other cases there may be three buds, in which case two are always on the right and one on the left).

Fifth day (Fig. 2c). The oozoid has almost completely degenerated. Usually the remnants of the oozoid are so heavily pigmented that it is impossible to identify whatever traces of the old intersiphonal band may still exist. Numerous reflecting cells arising from the disintegration of the intersiphonal band of the oozoid are now circulating freely in the blood stream and these can be traced as they move through the blood channels surrounding the siphons and in the area be-

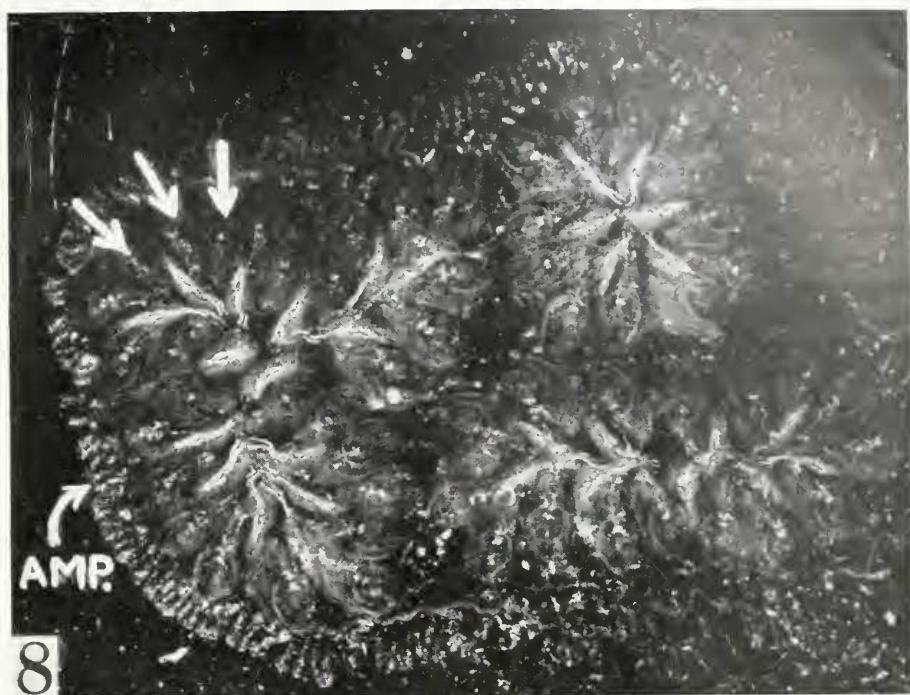
⁴Ampullae are terminal enlargements of the colonial blood vessels. Eight of them are present in the oozoid (Grave and Woodbridge, 1924); these progressively increase in number during the formation of a colony. In an adult colony they are found nearly everywhere, but are situated principally at the periphery. Several functions are attributed to them by Bancroft (1899): they act as storage reservoirs for the blood, they aid in blood propulsion since they are contractile, they act as organs for the secretion of test matrix and they probably function as respiratory structures.

PLATE II

All figures are photographs of the dorsal surface of the colonies taken by reflected light. Magnification *ca.* 7 \times .

FIGURE 8. Colony 11. Age 33 days. 6Z zooids are in maximum and permanent contraction and their intersiphonal bands are disintegrating. Reflecting pigment cells are accumulating in the ampullae at the periphery. 7Z zooids are appearing between the ampullae and the degenerating zooids and the oral extremities of their intersiphonal bands are forming (arrows). This is the second stage in the destruction of the intersiphonal patterns. On the 34th day the 7Z zooids are well formed with distinct intersiphonal bands.

FIGURE 9. Colony 35. Age 33 days. 7Z zooids are functioning and their intersiphonal bands are becoming double. Note the remains of the 6Z zooids at the center of the systems. Note also that reflecting pigment cells have almost completely disappeared from the peripheral ampullae.



8



9

tween the siphons of the first blastozoid. Simultaneously the intersiphonal band of pigment cells in the first blastozoid rapidly becomes more distinct by the addition of more reflecting cells in this region. Typically a solid band of reflecting cells is established between the two siphons; this band may be almost rectangular in shape, or it may be slightly irregular. Within the band there may be one or even two spaces free from reflecting cells. Some of the reflecting cells from the degenerating intersiphonal band of the oozoid can also be followed as they are carried through the blood channels of the second generation buds. While these buds remain small the circulating pigment cells may become trapped in them temporarily which accounts for the presence of a few scattered reflecting cells early in the establishment of each generation of buds; these tend to disappear later when blood channels enlarge allowing them to move on.

In order to compare the changes in the other colonies with those described for colony No. 38 reference should be made constantly to Table I which summarizes the relationship between changes in the intersiphonal bands of pigment cells and the major changes involved in asexual reproduction in all colonies studied. For example, on the fifth day the oozoid is undergoing degeneration in 23 colonies (i.e., in 59 per cent of all colonies). In ten of these reflecting cells are contained in the vascular channels and ampullae; circulating reflecting cells are also visible in two other colonies in which the oozoids are completely destroyed. Thus pigment cells are circulating in 12 colonies (30.7 per cent). In 13 colonies (33.3 per cent) intersiphonal bands are forming in first generation blastozoids and they are all single bands (in the other 26 colonies the reflecting cells in the first generation blastozoids are too scattered to constitute a definite intersiphonal band). Second generation buds are beginning to grow in 23 colonies (59 per cent). In general the observations given below refer only to colony No. 38; in order to ascertain how characteristic these changes are for all the colonies reference must be made to Table I.

Sixth day (Fig. 2d). All traces of the oozoid have disappeared but some reflecting cells are still present in the ampullae. The intersiphonal band in the 1Z zoid is well formed; within it there is one space free from reflecting cells. In several other colonies one or two spots free from reflecting cells are now visible within the intersiphonal bands, and in two colonies this tendency for reflecting cells to disappear from the midline of the intersiphonal band is so marked that the band is now classified as double (Table I). The 2Z buds are growing, and in some colonies the 2Z buds are sufficiently developed that they in turn bear buds which will become the third generation (3Z) zooids.

Seventh day (Fig. 2e). A second space free from reflecting cells has appeared in the midst of the intersiphonal band of the 1Z zoid. Oral siphons are beginning to appear in the 2Z zooids; some scattered reflecting cells are also present in these zooids. 3Z buds are present. Reference to Table I shows that very few oozoids are actually undergoing degeneration at this time and that reflecting cells are circulating in very few colonies. Perhaps the most striking change in the appearance of the intersiphonal bands is clearly demonstrated in Table I, viz., that in many colonies the intersiphonal bands which were originally single are now definitely becoming double due to the disappearance or rearrangement of reflecting cells at one, two, three or four spots along the length of the band.

Eighth day (Fig. 2f). The intersiphonal band tends to be almost double throughout its length. Typically the disappearance of reflecting cells spreads inwards from each end of the band, so that the last part of the band to become double is the part midway between the two siphons. Oral siphons are distinctly present in 2Z buds, and in some colonies the atrial siphons have also appeared (see Table I). Reflecting cells are present in many second generation buds, but they are seldom restricted sharply to the siphonal areas.

Destruction of Intersiphonal Bands in First Generation Blastozooids and Their Formation in Second Generation Zooids; Establishment of First Intersiphonal Patterns

Ninth day (Fig. 2g). The first generation blastozooid is undergoing degeneration; reflecting cells are circulating vigorously. In all colonies where 1Z zooids are degenerating reflecting cells are circulating (Table I). The 2Z zooids have grown rapidly; they possess both oral and atrial siphons and their intersiphonal bands are well developed; three clear spots are present in the band of one zooid and two in the other. The buds which will develop into the third generation zooids are just beginning to enlarge. A few scattered reflecting cells are appearing in these 3Z buds.

Tenth day (Fig. 2h). All traces of the first generation blastozooid have disappeared; however, reflecting cells are still present in the ampullae. The degeneration of 1Z zooids is still underway in many colonies (Table I). Although the two atrial siphons of the two 2Z zooids are only closely approximated in colony No. 38 they have fused in nine colonies to form a common cloaca, thus establishing a system of zooids for the first time in the life history of these colonies. In one of these colonies consisting of three zooids two atrial siphons have fused, but the third one is still separate. In some colonies the buds which will develop into the fourth generation zooids have already made their appearance (Table I).

Eleventh day (Fig. 2i). Reflecting cells are no longer present in the circulatory system of most colonies. The intersiphonal bands of the 2Z zooids are almost completely double, especially at the atrial end of the bands. The atrial siphons of the 2Z zooids have now fused to establish a common cloaca. Therefore the individual intersiphonal bands are now united at their atrial ends for the first time to form a true intersiphonal pattern. The buds which will develop into the third generation blastozooids are enlarging; in many colonies these buds contain scattered reflecting cells with no special distribution. The buds which will become the fourth generation zooids are now present.

Twelfth day (Fig. 2j). The intersiphonal bands continue to approach the condition of completely double bands, especially at their atrial ends; consequently the intersiphonal pigment patterns tend to consist of double bands in each blastozooid (as in Pl. I, Figs. 3 and 5). In four colonies one or more of the 2Z zooids are beginning to degenerate prematurely; in two of these the 2Z zooids had first joined a common cloaca before degeneration began (in contrast to Pizon, 1893, 1899a, and 1900, who believes that one of the primary causes of premature degeneration of zooids is the failure to join a common cloaca with other zooids). Oral siphons are appearing in 3Z buds; 4Z buds are present, but are not growing.

Destruction of Intersiphonal Bands in Second Generation Blastozooids and Their Formation in Third Generation Zooids

Thirteenth day (Fig. 2k). 2Z zooids with almost completely double bands are still present, but reflecting cells are appearing in the circulatory system. The 2Z zooids are beginning to degenerate in a number of colonies; in some only one or two zooids are degenerating while others are still normal; in several the zooids are strongly contracted, but actual degeneration has not yet begun. 3Z zooids are considerably larger, oral siphons are present in them, and scattered reflecting cells are also visible, especially around the oral siphons. 4Z buds have not yet started to grow.

Fourteenth day (Fig. 2l). Second generation zooids are undergoing degeneration, as is the case in many other colonies (Table I). In two colonies only one of the second generation zooids has started to degenerate; in another colony one of the 3Z zooids is also degenerating. The 3Z zooids have their siphons well formed and the atrial siphons have already fused to form a common cloaca. The intersiphonal bands are well developed; some of them are nearly double from their earliest appearance. The 4Z buds are enlarging.

Fifteenth day (Fig. 2m). The 2Z zooids are completely absent although they are still undergoing degeneration in several other colonies. Almost no change has occurred in the appearance of the intersiphonal bands. The buds which will become the fourth generation zooids are growing rapidly and 5Z buds have now appeared.

Sixteenth-seventeenth days (Fig. 2n). The 3Z zooids form a well-defined system with almost completely double intersiphonal bands of pigment cells. One colony is striking in that the band is double in one of the two zooids, but practically non-existent in the other. Oral siphons are just appearing in 4Z buds. In two colonies some of the 3Z zooids are degenerating prematurely.

Destruction of Intersiphonal Bands in Third Generation Blastozooids and Their Formation in Fourth Generation Zooids

Eighteenth day (Fig. 2o). 3Z zooids are undergoing degeneration and reflecting cells are circulating. Intersiphonal bands are forming rapidly in 4Z zooids, and they are double from their earliest appearance. The atrial siphons of the 4Z zooids have not yet joined to form a common cloaca. 5Z buds are beginning to grow.

Nineteenth day (Fig. 2p). All traces of the 3Z zooids are now lacking. The atrial siphons of the 4Z zooids have not yet joined for cloaca formation. 5Z buds are growing rapidly and 6Z buds have appeared.

Twentieth-twenty-first days (Fig. 2q). Beyond the nineteenth day daily observations were made only on ten colonies (9c, 11, 13, 23, 30b, 35, 38, 41, 44a and 46b); therefore the observations summarized in Table I beginning with the twentieth day are based only on these ten colonies unless otherwise indicated. Colony No. 38 has well developed 4Z zooids; a distinct intersiphonal pattern is now present since the atrial siphons have joined to form a common cloaca with the almost completely double intersiphonal bands radiating outwards from it.

Destruction of Intersiphonal Bands in Fourth Generation Blastozooids and Their Formation in Fifth Generation Zooids; First Appearance of More Than One Intersiphonal Pattern in a Colony

Twenty-second day (Fig. 2r). 4Z zooids are undergoing degeneration; reflecting cells are circulating strongly. The 5Z zooids do not possess atrial siphons as yet; their intersiphonal bands are almost completely double from their first appearance. These fifth generation zooids are distributed into three groups indicative of the three systems of zooids which they will constitute later; two groups of four zooids each are separated somewhat from the remaining zooids which, though widely separated from each other, are destined to form only one system.

Twenty-third–twenty-sixth days (Fig. 2s). 5Z zooids are now well developed; all the intersiphonal bands are completely double and are radiating outwards from three common cloacas, i.e., the colony now consists of three systems of zooids and therefore of three intersiphonal patterns. 6Z buds are enlarging.

Destruction of Intersiphonal Bands in Fifth Generation Blastozooids and Their Formation in Sixth Generation Zooids

Twenty-seventh day (Fig. 2t). The 5Z zooids are undergoing degeneration and reflecting cells are circulating; all the thirty-two 6Z zooids have had double intersiphonal bands almost from their earliest appearance. These zooids are distributed in four groups indicative of the four systems they will establish, although no common cloacas have yet formed; zooids are distributed in groups of seven, eight, eight and nine.

Twenty-eighth–thirty-first days (Fig. 2u). Four well-defined systems of 6Z zooids are now present, each with a common cloaca and an intersiphonal pattern. The observations recorded in Table I for the twenty-ninth day are based on all 39 colonies, i.e., on the 10 colonies on which daily observations have been made, as well as on the 29 colonies which have not been examined since August 19. The observations recorded beyond the twenty-ninth day are again based only on ten colonies.

Destruction of Intersiphonal Bands in Sixth Generation Blastozooids and Their Formation in Seventh Generation Zooids

Thirty-second day (Fig. 2v). Colony No. 38 has changed from the sixth to the seventh generation of zooids; all of the new intersiphonal bands are almost completely double; the zooids are already arranging themselves into eight groups, but only a few atrial siphons have fused as yet. There is a total of 61 zooids.

Thirty-third–thirty-fifth days (Fig. 2w). The 7Z zooids are now arranged in eight distinct systems, each with its distinct intersiphonal pattern consisting of double intersiphonal bands.

DISCUSSION

Literature

Attention has frequently been called to the brilliant and varied coloring of the flower-like groups of ascidiozooids which constitute Botryllus colonies (M'Intosh, 1901; Herdman, 1925). According to Webb (1939), "It appears that all brightly

coloured species owe their colour to pigment cells which are morphologically blood cells, though in most cases they do not participate much in the general circulation but become more or less permanently lodged along the walls of the peripheral vessels" (p. 505). For the most part, however, only incidental observations have been recorded concerning intersiphonal bands of pigment cells. Pallas (1774) illustrates several *Botryllus* systems in his Plate IV, Figs. 2, 3, 4 and 5, and double intersiphonal bands are clearly indicated. Krohn (1869b) in his explanation of his Plate XIV, Fig. 2 calls attention to "Breiter kreideweisser Pigmentstreifen zwischen den beiden Leibesöffnungen." Van Name (1910) states that "During life, . . . , the zooids, especially their anterior ends and dorsal portions, and the bulbs of the test vessels, are marked with a light-colored pigment, which mostly disappears after death" (p. 352). In more detailed descriptions of *Botryllus* colonies the presence of some sort of intersiphonal bands has been noted in numerous instances. This is especially true of *Botryllus violaceus* (Milne-Edwards, 1842). In this species (?) Giard (1872) notes (p. 621), "Les deux orifices sont reliés entre eux par des lignes plus ou moins larges, d'une coloration qui varie entre le blanc pur et le jaune brunâtre en passant par toutes les teintes intermédiaires." Concerning this same species (?) Alder and Hancock (1912) state (p. 70), "the thorax from near that [oral] aperture downwards [is] marked with a double line of opaque white, broadest above and ending on the margin of the tubular common orifice; these lines [are] sometimes yellowish and occasionally broader and confined more exclusively to the base." In *Botryllus smaragdus* Giard (1872) mentions the presence of "Lignes radiales d'un jaune plus ou moins vif, parfois à peine indiquées" (p. 626) and Pizon (1899a) describes "des lignes radiales jaunes qui s'étendent, avec une largeur et des nuances très variables, sur la face supérieure de la branchie" (p. 396). Similar radial lines extending from the oral to the atrial siphons have been described by Giard (1872) in *Botryllus auro-lineatus* and *Botryllus morio*, by Alder and Hancock (1912) in *Botryllus miniatus* and by Van Name (1931) in *Botryllus primigenus*. Of these authors only Pizon definitely states that the pigment granules constituting the intersiphonal bands are contained within special cells called chromatocytes, and that these granules are about $1\ \mu$ in size and are animated by Brownian movement. Van Name (1902) also notes that in the closely related *Botrylloides nigrum* similar white pigment is contained in opaque oval cells of the same size as those containing the ground color.

The only extensive observations concerning intersiphonal bands in *Botryllus* are recorded by Bancroft (1903). He refers to these bands in one of two ways: as dorsal double bands, or as two parallel white bands extending from cloaca to branchial orifice; he also speaks of the blastozooids possessing such bands as white-striped zooids. Although he does not state so specifically he realizes that the pigment involved is contained in specific pigment cells. He believes that the position of these bands does not depend on that of blood vessels. His paper deals to a considerable extent with color variations in *Botryllus*. His comments concerning variations in intersiphonal bands will be incorporated into the discussion below.

Repeated Destruction and Formation of Intersiphonal Bands of Pigment Cells During the Development of a Colony

The intersiphonal band which develops in the oozoid is usually a solid band of cells between oral and atrial siphons. This band arises rather rapidly during

metamorphosis of the tadpole and undergoes few changes while the oozoid persists, except that it sometimes tends to become double at its oral extremity. This transformation of single intersiphonal bands into double bands occurs quite slowly in the oozoids and is rarely completed before the oozoid degenerates, so that single or at best partially double intersiphonal bands are characteristic of oozoids at their highest state of development. By the third day after attachment the first generation blastozoid is growing rapidly and scattered reflecting cells are present in it. By the fourth day both of its siphons are indicated and its reflecting cells are quite definitely restricted to the region immediately surrounding the siphons, with a few scattered cells between the siphons. The oozoid then begins to degenerate and the intersiphonal band of the oozoid is destroyed. The pigment cells set free from the intersiphonal band are then present in the circulatory channels and are carried passively by the blood stream. Such degenerative changes occur in the oozoids of the majority of colonies on the fifth day (Table I). Simultaneously intersiphonal bands are forming in the first generation blastozoids, and they are all single bands. 2Z zooids are just beginning to grow rapidly, but they possess neither oral nor atrial siphons as yet. 3Z buds are not present. On the sixth day fewer oozoids are degenerating; most of the colonies have single intersiphonal bands forming in 1Z zooids, but in a few colonies these bands are transforming into double bands. 2Z zooids are growing rapidly, although they still possess neither oral nor atrial siphons. 3Z buds are now appearing. By the seventh day degeneration of oozoids is nearly completed and reflecting cells are circulating in few colonies. The most striking change has been the transformation of almost all single intersiphonal bands into double bands. Oral siphons are just appearing in 2Z zooids and 3Z buds are now present in almost all colonies. By the eighth day the first generation blastozoids are in their optimal state of development. The characteristics of this highly developed condition are as follows: (a) almost no reflecting cells are circulating, indicating that oozoids have all degenerated some time ago and that first generation blastozoids have not yet started to degenerate; (b) the intersiphonal bands of first generation blastozoids are almost all double, a condition which is usually realized only when the zooids are fully developed; (c) all 2Z zooids are growing rapidly and both oral and atrial siphons are forming in them; (d) 3Z buds are present in all colonies.

Then, on the ninth day the 1Z zooids begin to degenerate, accompanied by the appearance of reflecting cells in the circulatory system once more, and accompanied also by the onset of rapid growth of 3Z zooids. Degeneration of first generation zooids and their intersiphonal bands is at a maximum on the tenth day. Intersiphonal bands are forming in 2Z zooids, and note especially that there are almost as many colonies with double bands as with single bands at this time. For the first time the atrial siphons of 2Z zooids are fusing in some colonies to establish a common cloaca and thus to establish a system of zooids within each colony, thereby allowing establishment of the first intersiphonal patterns. 4Z buds are beginning to appear. By the twelfth day the 2Z zooids have attained their maximum development. The characteristics of this highly developed condition of the colonies are the same as those when 1Z zooids were best developed, viz., few reflecting cells are circulating, almost all intersiphonal bands are double, all 3Z zooids are growing rapidly, and 4Z zooids are present in almost all colonies. In addition atrial siphons of 2Z zooids have fused to form common cloacas in most colonies.

Degeneration of 2Z zooids begins on the thirteenth day, reaching its maximum on the fourteenth day. Most of the intersiphonal bands forming in 3Z zooids are single at this time. The atrial siphons of 3Z zooids are beginning to fuse to establish common cloacas. 3Z zooids reach their maximum development on the seventeenth day. The new intersiphonal pattern characteristic of the colony at this time is more complex than previously since it consists of more intersiphonal bands. Again the characteristics of the colonies at this moment of maximum development are the same as at the time of maximum development of 1Z and 2Z zooids.

3Z zooids then begin to degenerate and 4Z zooids attain their maximum development on the twenty-first day. Then the 4Z zooids degenerate and 5Z zooids reach their maximum development on the twenty-sixth day. These in turn degenerate and 6Z zooids attain maximum development on the thirty-first day. The 6Z zooids then degenerate and 7Z zooids are in their most highly developed condition on the thirty-fifth day. 5Z, 6Z and 7Z zooids are characterized by double intersiphonal bands almost from their earliest appearance. Thus under laboratory conditions a period of 4 to 5 days elapses between the highest state of development of one generation of zooids (and its intersiphonal bands) and the highest state of development of the next generation (and its intersiphonal bands).⁵

During each brief period of four to five days the intersiphonal bands undergo an orderly series of stepwise transformations. a. The pigment cells which constitute a new intersiphonal band first appear near the oral siphons. They are fairly scattered at first, but very soon they form a solid band of cells in the dorsal midline extending from oral to atrial siphons. b. The originally solid bands of reflecting cells then begin to separate lengthwise so that each intersiphonal band shows indications of becoming double throughout its length (Pl. II, Fig. 9). These bands progressively become more completely double (Pl. I, Fig. 4) until they are distinctly double (Pl. I, Fig. 3). The pigment cells then reflect light to the maximal extent and the particular generation of zooids involved has then attained its highest state of development. Bancroft (1903) also recognized that these intersiphonal bands are progressively built up. Pizon (1899a) realized that the intersiphonal bands change with time, but he gave no description of the changes. c. The most radical change in the intersiphonal patterns then occurs. They assume a very irregular appearance (Pl. I, Fig. 6) as compared with their extremely regular appearance at the time of their greatest development (Pl. I, Figs. 3 and 5). This irregularity is due to the contraction of some of the intersiphonal bands, due in turn to the contraction of the zooids involved. At a given instant some intersiphonal bands are almost completely contracted, others only partially, but an instant later different bands become contracted and those formerly contracted are then somewhat relaxed, with the result that the intersiphonal patterns are rapidly changing their configuration. The intersiphonal bands then undergo maximum and permanent contraction (Pl. II, Fig. 8), and at this time the intersiphonal bands are destroyed, i.e., the reflecting pigment cells constituting them are released from the

⁵ This period of four to five days between the maximum development of successive generations of zooids agrees with the intervals established by Berrill (1935a). However, it should be emphasized, as Bancroft (1903) and Grave (1933) have already done, that growth of *Botryllus* is less vigorous in aquaria than in its natural habitat. In colonies growing in the Eel Pond, Grave observed that the interval between successive generations is reduced to 2 or 3 days.

band and pass into the blood channels between and around the siphons and degeneration of the zooids is underway. These circulating pigment cells are swept quickly into the ampullae (amp., Pl. II, Fig. 8) at the periphery of the colony and into the blood channels of the next generation blastozooids and can be traced as they circulate around and between the siphons of these new zooids. These reflecting pigment cells sometimes begin to circulate even before the atrial siphons have appeared in the new generation zooids, and they are usually circulating strongly when the degenerating zooids are still of adult size, are three-fourths that size, are one-half as large as adults, and even when they are completely reduced.

While the old intersiphonal bands are undergoing destruction new intersiphonal bands are forming in the new generation zooids (Pl. II, Fig. 8, arrows). Because of the almost perfect coincidence in the time of destruction of the intersiphonal bands in one generation of zooids and their establishment in the next generation it seemed certain that some of the circulating reflecting cells must become secondarily attached in the intersiphonal region of the next generation of zooids to participate in the formation of its intersiphonal bands. This seemed all the more certain in view of the fact that during the final rapid development of these new bands of pigment cells the reflecting cells disappear almost completely from the circulatory system (compare peripheral regions of Pl. II, Figs. 8 and 9). However, in some exceptional cases (Pl. I, Fig. 7) it is evident that new intersiphonal bands (arrows) can originate even before contraction and subsequent destruction of the bands of the old generation zooids has begun. This suggests that the first reflecting cells to appear in the intersiphonal region of the new zooids arise *in situ*. Whether all the pigment cells of the new bands arise locally, or whether they are augmented with cells from the intersiphonal bands of the degenerating zooids can be determined only by experimentally preventing the reflecting cells from the degenerating pigment bands from entering the new generation zooids, as well as by a careful histological study. Bancroft (1903) has recorded an observation of interest in this connection. In his studies on the fusion of Botryllus colonies he has observed that a single system may contain zooids derived from two different colonies; the zooids from one colony may possess well developed double intersiphonal bands whereas those of the other may possess no visible bands (Pl. XVII, Fig. 23). In his discussion (pp. 174-175) he states that "In the fused colonies the zooids may differ in color; and not the slightest tendency toward an equalization of this difference could be detected." This evidence would suggest that intersiphonal bands arise *in situ* or not at all; it suggests that even if reflecting pigment cells are present in part of a colony they can never participate in the formation of a pigment band in zooids which do not inherently possess one. If the reflecting cells from the degenerating pigment bands are not involved in the establishment of new intersiphonal patterns, perhaps they too are used as food material by the newly forming zooids; it is known that all other cells arising from degeneration of the zooids are so utilized.

Eventually the number of zooids in a colony has increased to the point where they can no longer gather around a single common cloaca. More than a single common cloaca then forms, and some zooids assemble around each with the result that the colony then consists of more than one system of zooids. Since the intersiphonal bands of one system constitute one intersiphonal pattern, the colony then has more than one intersiphonal pattern. As more and more generations of zooids develop the number of systems, and therefore the number of intersiphonal patterns,

progressively increases. Krohn (1869a and b) clearly recognized that as each new generation of buds matures, the old systems are constantly replaced by new ones which usually correspond with the parent system neither in their form nor in the number of single individuals composing them.

Thus it is evident that any *Botryllus* colony is characterized by a specific intersiphonal pattern or group of patterns for only a very brief period. Any given intersiphonal pattern is then rapidly destroyed within a few hours and an entirely different pattern or group of patterns is built up, only to be destroyed in turn. Moreover, it is evident that this progressive remodeling of the intersiphonal patterns within a given colony is intimately associated with the process of asexual reproduction, so intimately in fact as to verify the statement by Berrill (1941c) to the effect that there is a "rigorous correlation of the features characterizing any given stage, so that if the developmental stage of one feature is known the stage as a whole can be accurately defined" (p. 100).

Variations in Intersiphonal Patterns When Different Colonies Are Compared

Some Colonies Lack Distinct Intersiphonal Patterns

Some colonies are collected in nature in which the intersiphonal patterns are not strongly expressed even when the zooids are in their maximum state of development. It is interesting to inquire whether this weakness in expression of the pattern in certain colonies is merely due to lack of proper environmental conditions, whether it is due to an unhealthy condition of the colony, whether it is a temporary condition characteristic only of that particular generation of zooids which is functioning at that instant, or whether it is a permanent characteristic of that particular colony appearing in all generations of zooids. If such a weakness of expression is a constant characteristic in certain colonies, perhaps it is genetically determined.

Two days after attachment of the larvae considerable variation is evident when the intersiphonal bands of different oozoids are compared. Some oozoids exhibit a *strong* intersiphonal band, i.e., there is a broad, almost solid band of reflecting cells between the two siphons; others possess a *medium* band, i.e., there is a rather narrow or irregular band consisting of relatively few reflecting cells; still others possess a *weak* intersiphonal band, i.e., there are only a few reflecting cells near the dorsal midline with no obvious arrangement into a band. In 15 of the colonies on the sixth day the reflecting cells are not concentrated into a distinct intersiphonal band in 1Z zooids. Of these, 11 were derived from oozoids originally classified as possessing *weak* bands and 4 from oozoids with *medium* bands; all of the oozoids whose intersiphonal bands were originally classified as *weak* (and not omitted from the observations recorded in this paper) are among these 15 colonies, but 6 colonies derived from oozoids whose bands were originally classified as *medium* are not in this group. No blastozooids derived from oozoids which exhibited *strong* intersiphonal bands are found in this group. On the eighth day 14 of the 15 colonies still do not have the reflecting cells arranged into a definite intersiphonal band, and no distinct intersiphonal band is formed before the 1Z zooids degenerate. By the eleventh day 25 colonies possess well developed intersiphonal bands in 2Z zooids, but such bands are lacking in 14 colonies. All of the colonies derived from oozoids originally possessing *weak* bands are among these 14, as

well as 3 from oozoids whose intersiphonal bands were originally classified as *medium*; not a single colony derived from an oozoid exhibiting *strong* intersiphonal bands is to be found in this group. Moreover no distinct bands appear in these 14 colonies before the 2Z zooids degenerate. On the sixteenth day, although distinct bands are present in 4Z zooids in 22 colonies, no such bands exist in 15 colonies, and all colonies originally exhibiting *weak* bands are included among these 15. On the twenty-ninth day three colonies originating from oozoids with *weak* intersiphonal bands possess no distinct intersiphonal bands in 5Z zooids; two colonies are undergoing the transformation from fifth to sixth generations and therefore the expression of the intersiphonal bands could not be determined; six possess no distinct bands in 6Z zooids. Two other 5Z colonies and three 6Z colonies are now classified as possessing no distinct intersiphonal patterns; these were derived from oozoids originally classified as possessing *medium* intersiphonal bands.

Thus all colonies in which only *weak* intersiphonal bands form in the oozoid stage form only *weak* bands throughout their development, at least through the seventh generation of blastozooids. This weakness of expression of the intersiphonal bands is therefore a constant characteristic throughout the developmental history of certain colonies. On the contrary, oozoids which possess well developed (*strong*) intersiphonal bands develop into colonies which always have well developed bands. This difference between *weak* and *strong* expression of intersiphonal bands is therefore a constant one and is probably genetically determined. In the present study no attempt was made to determine whether the tadpoles which give rise to oozoids with *weak* intersiphonal bands are derived only from adult colonies in which no distinct bands occur, or whether they can come from adults possessing distinct bands. However, Bancroft (1903) has recorded some observations of interest in this connection. He collected tadpoles from three mother colonies. The tadpoles from the first mother colony gave rise to a large number of colonies called Family I; tadpoles from the second mother colony established Family II; and Family III originated from tadpoles which emerged from the third mother colony. He observed that double bands formed only in Family II and Family III, and the mother colonies of both these families had well developed dorsal bands. He also noted that in Family II two colonies developed dorsal double bands, whereas two had no trace of them. This may indicate that colonies with *strong* intersiphonal bands can be established only from tadpoles which emerge from mother colonies with well developed bands; however, colonies with *weak* intersiphonal bands can also come from tadpoles emerging from colonies with well developed bands. Should there prove to be a genetic basis for this difference between *strong* and *weak* expression of intersiphonal bands, it appears likely that the *strong* expression will prove to be dominant.

The category of *medium* as applied to the classification of intersiphonal bands appears to be an artificial one, since colonies derived from oozoids classified as *medium* are subsequently classified in part with the group lacking distinct bands in later generations, but mostly with the group possessing such bands. Thus the group of oozoids classified as *medium* is probably a heterogeneous group, part of its members actually belonging among the *weak* group, but showing the maximum development of the bands to be found in this group, and the rest belonging to the *strong* group, but showing only a minimal development of the bands. The classification of *medium* is therefore discarded as of no significance.

The Number of Zooids in a Given Generation Is Not Constant

Due to differences in the number of buds per zooid

Usually throughout the developmental history of any colony the number of zooids progressively increases and accordingly as each new generation of zooids becomes mature, the intersiphonal pattern of the colony becomes more complex than when the previous generation of zooids was present. However, the complexity of the pattern is not the same in all colonies even when they are in the same stage of development. This is evident if the intersiphonal patterns of three of the colonies illustrated in Plate 1 are compared. These 3 colonies are all in the same stage of development, i.e., sixth generation zooids are functioning in each, but in the colony illustrated in Figure 3 there are only 5 zooids in the 6Z generation, in the colony illustrated in Figure 4 there are twelve 6Z zooids, while in the colony illustrated by Figure 5 there are twenty-three 6Z zooids. Obviously even when colonies of the same stage of development are compared variations in their intersiphonal patterns still exist due to differences in the number of zooids, which is due mainly to differences in the rate of budding in different colonies. A question then arises concerning the factors which determine the number of buds produced by any zooid. Is the number of buds produced by a single zooid purely a fortuitous response to the environment, varying from generation to generation within any given colony? Or, is the number of buds produced by each zooid constant from generation to generation in a given colony and variable only when different colonies are compared? Any regularity in budding within one colony may possibly indicate that the budding pattern is a genetic characteristic of a colony.

According to Pizon (1893) both lateral walls of the atrium of the tadpole undergo a localized thickening sometime before the tadpole escapes from the parent. Such a thickening is the first step in the development of an asexual bud. Thus budding in the oozoid begins bilaterally, but the thickening on the left is always much less accentuated than the one on the right. By the time the tadpole has escaped from the parent the bud on the left has entirely disappeared, whereas the one on the right has developed sufficiently that the beginnings of a pharynx and two atrial sacs are recognizable. He notes that the intestine in the oozoid is crowded to the left and he believes that the pressure exerted by the intestine against the atrial wall most likely inhibits the morphogenesis of the left bud at an early stage. Most ascidiologists agree that the oozoid gives rise to a single bud on its right side, although Giard (1872) and Jourdain (1886) believe that the oozoid generally produces two first generation blastozooids.

According to Herdman (1925) bilateral budding is then the rule in the blastozooids, so that theoretically the colony should consist of a number of zooids represented by some power of two. Pizon (1899a and 1900) also regards as normal the production of two buds, one on the right and one on the left of the parent, although in many instances only one bud develops, and in these cases he states that the single bud may be either on the right or left side (1893). However, Berrill (1941b) has noted that there is usually a size difference between bud primordia on the right and left sides, the one on the right being the larger. Moreover, Oka (1892) states that whenever an equal number of buds is developing on the right and left sides of the parent those on the left are always somewhat retarded. It is therefore not surprising that Oka finds that whenever a single bud

is produced, it is always on the right side. Whenever one bud is lacking Pizon always finds a feeble diverticulum of the atrial wall where that bud should have developed, indicating that budding of the blastozoid is always bilateral initially regardless of whether both buds actually continue to develop. Krohn (1869a) and Della Valle (1882) on the contrary regard the production of a single bud as the rule among blastozoids and the production of more than one bud per zooid as the exception. However, Krohn recognizes that two buds can form, in which case one is always on the right and the other on the left.

Oka (1892) has also observed that many times the original atrial thickening is not entirely used up in the production of one bud leaving a portion of the budding zone unused. Sometimes this remnant of budding material gives rise to a second bud on that same side of the parent; such a second bud is always younger in its developmental stage than the first bud developed on that side of the body. Della Valle (1882) also recognized that when more than one bud develops on the same side of the body the first one to appear is always in advance of the second in its stage of development. Pizon (1893) has observed one young colony of *Botryllus* in which each blastozoid carried two buds on each side; he states that the same peribranchial thickening on each side produced the two adjacent diverticula. Oka (1892) records that in the *Botryllus* colonies found along Japanese coasts it is not unusual to find zooids producing as many as six asexual buds. If an even number of buds is produced, there will be the same number of buds on each side of the body; if an odd number is formed, there is always one more on the right than on the left. According to Berrill (1941c) double buds are more likely to develop in early generations of zooids before sexual reproduction begins as well as in later generations when large zooids capable of reproducing sexually are present, rather than in intermediate stages.

Thus it is obvious that there are bilateral bud primordia in both the oozoids and blastozoids, but that there is an asymmetry of budding capacity with the right side usually the more productive. Most of the above information concerning the budding pattern is based on isolated observations or on a study of a very small number of colonies. It seems worthwhile to follow the budding patterns of the colonies studied in this paper in an attempt to determine whether any generalities concerning the budding pattern can be formulated.

When all the colonies are examined on the sixth day of development 6 possess only one 2Z bud (2ZR), 21 possess two 2Z buds (2ZR and 2ZL), and 12 possess three 2Z buds, two on the right side (2ZR and 2ZR') and one on the left (2ZL). When the second generation zooids are examined on the tenth day to determine the number of 3Z buds per zooid, it is noted that in some colonies each zooid produces only one 3Z bud; in others some zooids produce one 3Z bud and others two such buds; in other colonies all zooids produce two buds each; in still others some zooids produce two 3Z buds and others three 3Z buds. In order to decide whether the number of 3Z buds per zooid in any given colony bears any relationship whatsoever to the number of 2Z buds per zooid earlier produced by the same colony, the data may be arranged advantageously in a checkerboard table (Table II). From this table it is evident that three of the six colonies (50 per cent) which produced only one 2Z bud per zooid also produce only one 3Z bud per zooid; moreover, an additional one of these six colonies (17 per cent) produces one 3Z bud per zooid in at least some of the zooids. Ten of the 21 colonies (48

TABLE II

Comparison of the number of buds per zooid in different generations of the same colonies

		No. of 3Z buds per zooid on tenth day				No. of 4Z buds per zooid on seventeenth day					Av. no. of 6Z zooids per colony on twenty-ninth day
		1 bud	1 bud or 2 buds	2 buds	2 buds or 3 buds	1 bud	1 bud or 2 buds	2 buds	2 buds or 3 buds	3 buds	
No. of 2Z buds per zooid on sixth day	1 bud	3 (50%)	1 (17%)	2 (33%)		1 (17%)	2 (33%)	3 (50%)			6.17
	2 buds	5 (24%)	3 (14%)	10 (48%)	3 (14%)	2 (9.5%)	10 (48%)	8 (38%)		1 (5%)	12.23
	3 buds		7 (58%)	2 (17%)	3 (25%)	3 (25%)	7 (58%)	1 (8%)	1 (8%)		20.90

per cent) which produced two 2Z buds per zooid also produce two 3Z buds per zooid; moreover, six additional colonies (28 per cent) produce two 3Z buds per zooid in at least some of their zooids. Thus, in these two groups of colonies there appears to be at least some slight tendency for colonies to produce the same number of second and third generation buds per zooid. However, those 12 colonies which originally produced three 2Z buds per zooid appear incapable of maintaining this rapid rate of budding, and tend to produce only two 3Z buds per zooid instead (in 17 per cent of these colonies there are two 3Z buds on each zooid, but in 58 per cent there are two 3Z buds on only some zooids and one 3Z bud on others, and in 25 per cent there are two 3Z buds on some zooids and three 3Z buds on others). Pizon (1899a) has noted a similar tendency towards reduction in the number of buds per zooid following a generation in which three buds per zooid was the rule, and he suggests that this reduction serves to compensate for the excessive budding of the earlier generation. In his case the reduction was to even less than two buds per zooid.

Similarly, in order to see whether the number of 4Z buds per zooid bears any relationship whatsoever to the number of 2Z buds per zooid produced by the same colonies these data may be added to the checkerboard table. In some colonies on the seventeenth day there is only a single 4Z bud per zooid; in others some zooids produce one bud, others two; in some colonies each zooid produces two 4Z buds; in others some of the zooids produce two 4Z buds, others three 4Z buds; in one colony each zooid produces three 4Z buds. Of the six colonies originally producing one 2Z bud per zooid only one colony (17 per cent) produces a single 4Z bud per zooid. Fifty per cent produce two buds per zooid, and an additional two colonies (33 per cent) produce two buds per zooid in at least some of their zooids. Thus, by the fourth generation produced by asexual budding these colonies appear to be approaching the ability to produce two buds per zooid. However, if each 4Z zooid then produced two 5Z buds, and each of these in turn produced two 6Z buds, these six colonies should average eight zooids in the sixth generation. Instead they actually average only 6.17 zooids, and therefore slightly less than two buds per zooid must be the rule in giving rise to fifth and sixth generation zooids.

Of the 21 colonies originally producing two 2Z buds per zooid eight (38 per cent) produce two 4Z buds per zooid, but ten (48 per cent) produce two 4Z buds on only some of their zooids. Thus the ability to produce two buds on each zooid is no longer maintained rigidly in these colonies. If each zooid did continue to produce two buds up to the 6Z generation, we would expect to find thirty-two 6Z zooids. Instead we find on the average only 12.23 6Z zooids, indicating a budding rate of about 1.5 buds per zooid beyond the formation of 3Z buds. Those twelve colonies originally capable of producing three 2Z buds per zooid have almost lost this ability by the fourth generation of blastozooids. Only one colony (8 per cent) produces three 4Z buds in some of its zooids; seven colonies (58 per cent) produce two 4Z buds in some of their zooids and in three colonies (25 per cent) only one 4Z bud per zooid is produced. Thus, in these colonies, less than two buds per zooid now seems to be the rule in producing fourth generation blastozooids. If two buds per zooid continued to form to give rise to 4Z, 5Z and 6Z generations, we would expect to find forty-eight 6Z zooids per colony; instead we find only 20.9 6Z zooids per colony on the average, or roughly a budding rate of 1.5 buds per zooid after the 3Z zooids are established.

Grave (1933) has emphasized that the growth rate of different *Botryllus* colonies varies greatly even in their normal environment, but he made no attempt to analyze these differences in terms of the number of buds produced per generation of zooids. He has observed one colony which developed from a single larva on June 20 to 3000 individuals on July 20; he states that the number of individuals in this colony doubled every two or three days. In this colony the number of buds per zooid must have been at least two to account for such rapid growth; however, he states explicitly that he selected as examples only the most proliferative colonies; therefore in most colonies the budding rate must be less than two buds per zooids.

Therefore, although colonies differ in the number of 2Z buds produced per zooid, this difference appears to be only temporary and is not maintained in later generations, at least not under laboratory conditions. Instead, in later generations all colonies tend to produce on the average 1.5 buds per zooid. Nevertheless, the initial difference in the number of 2Z buds per zooid is sufficiently great that even though the budding rate then tends to become equalized in all colonies, the number of zooids present in the 6Z generation will be small in colonies which originally produced only one 2Z bud per zooid (6.17), will be about twice as great in colonies originally producing two 2Z buds per zooid (12.23), and will be about three times as large in colonies originally producing three 2Z buds per zooid (20.9). Consequently the number of 2Z buds per zooid is an important factor in accounting for differences in the intersiphonal patterns in later generations even when colonies in the same stage of development are compared. The factors which account for the differences in vigor, which in turn allow one, two, or three 2Z buds to form on each zooid, are not known. If they are due to genetical differences the action of heredity in controlling the number of buds per zooid appears to be limited to controlling bud formation only in the establishment of this one generation. These observations seem to be based on a study of more colonies than those examined by Berrill (1935c); his observation that the first bud (1Z) gives rise to a second bud (2Z) and that to a third bud (3Z) and the third to a bud on each side may hold for

a particular colony, but it is certainly not the characteristic budding pattern of the majority of colonies.

Due to premature atrophy of developing buds

In addition to differences in the actual number of buds the possibility that some of these buds may degenerate prematurely before reaching the adult stage must also be considered as a limiting factor in controlling the number of zooids in a colony and therefore the complexity of the intersiphonal patterns of the colony. Pizon (1899a) has observed that frequently a considerable number of buds are arrested in their development and begin to regress. Some disappear very early, at the moment when they are still small simple vesicles which are beginning to produce herniations on the flanks of their parents. Others regress much later when they have already attained one-fifth to one-fourth adult size. Regression even occurs prematurely in some large zooids which have the internal organs almost completely formed.

Pizon (1893, 1899a and 1900) regards the failure of the bud primordia to produce buds as well as the premature atrophy of such buds once they have started to develop as due primarily to nutritive deficiencies. Abortions occur when buds are too closely pressed against the older zooids or against the substrate of the colony, thereby constricting the blood channels and preventing the entrance of the blood stream from parent into bud. Moreover he notes that such atrophy is more frequent in colonies in early stages of formation and suggests that this may be due to the fact that at first all of the energies of the newly forming colony are directed toward elaboration of the test with little left over to enable buds to develop profusely. He particularly emphasizes that isolated zooids or very small systems are more likely to atrophy than large systems, and he suggests that it is necessary that a zooid participate in the organization of a system if it is to survive. Oka (1892) also suggests that crowding or lack of it probably determines whether or not a bud will develop to maturity, but he emphasizes that the left bud primordium is more sensitive to adverse conditions than the right, and is less likely to survive. However, there are other examples of premature degeneration where no apparent causes are to be discerned. During the first seven generations of blastogenesis studied in this present paper there have been few examples of premature atrophy of zooids once they have started to develop. Where such atrophy occurred there was no indication of crowding. Moreover, as noted earlier (p. 83), some zooids degenerate prematurely even after they have become adults and even though they are associated with other zooids within a fairly large system, so participation in system formation is not in itself a guarantee that premature degeneration will not occur.

Due to persistence of parent zooids after their buds attain the adult state

Another factor which can affect the number of zooids present in a colony at a given stage of development is whether the parent zooids always degenerate as soon as their buds become capable of functioning, or whether they sometimes persist to function simultaneously with their buds. The results recorded in this paper are in complete agreement with those of Pizon (1893) who states that when one generation attains the adult state, the preceding generation degenerates, and that two generations of zooids never arrive at the adult state simultaneously. However, several authors (Delle Valle, 1882; Bancroft, 1903; and Berrill, 1935b) have re-

ported that two generations of zooids can function side by side. If this actually does happen, the number of zooids and accordingly the complexity of the intersiphonal patterns would be correspondingly increased.

Due to fusion of adjacent colonies

Yet another factor which could influence the number of zooids in a colony and accordingly the intersiphonal patterns is the possibility that adjacent oozoids or older colonies may fuse indistinguishably. No such fusion was witnessed in the present work because every effort was made to prevent it by destroying adjacent colonies whenever there was a possibility of their fusing. Herdman (1925) suggests that large colonies need not necessarily arise from a single oozoid since several larvae may attach close together and form colonies which fuse so indistinguishably that even within one system some zooids may be derivatives of one oozoid, whereas the others come from another oozoid. Pizon (1900) has recorded an unquestionable case of such fusion. Bancroft (1903) has carried out an extensive study of the process of fusion and he has demonstrated that although unrelated colonies generally do not fuse, fusion can occur between some of the larvae derived from the same mother colony providing they become attached close together. Thus, any colony whose developmental history is unknown may conceivably be the derivative of more than one oozoid.

The Formation and Geometrical Form of Systems and of Intersiphonal Patterns

As indicated in the introduction each system consists of from 2 to 23 blastozooids, with an average of 8.3 blastozooids per system in 200 systems examined in colonies collected at Woods Hole. Obviously the intersiphonal pattern will vary according to the number of zooids involved in its formation. There is no opportunity for a system to form until the second generation zooids are well developed, since only a single zooid is present in the oozoid and first generation blastozooid stages. However, if there are two or more second generation or later generation zooids their atrial extremities approach one another and fusion of the separate atrial openings occurs so that the colony then possesses a central common cloaca about which the zooids are radially arranged with their separate oral siphons at the periphery. The originally separate intersiphonal bands then radiate from the common cloaca and the net result is the first establishment of an intersiphonal pattern. Sometimes, especially in older colonies, the zooids have to move through considerable distance to participate in the establishment of a system. Pizon (1899a) has observed zooids in less than one day make half to almost a complete turn in order to bring their atrial extremities into contact with those of other zooids. Sometimes the formation of a common cloaca occurs in stepwise fashion, i.e., two or more zooids may form one common cloaca, and several may form another, but later these two cloacas fuse to become a single opening. Bancroft (1903) states that there is evidently some attractive force, the nature of which is entirely unknown, which causes zooids to join into a system.

There appears to be a maximum number of zooids which can constitute a single system. According to Pizon (1899b) and Herdman (1925) when blastozooids become too crowded against each other and no space remains for additional zooids

around a single cloaca one or more blastozooids become crowded outwards away from the cloaca and come to lie at the periphery, each such isolated zooid constituting a starting point for the formation of an additional system. Oka (1892) also emphasizes that when additional systems are first established they contain only a small number of individuals, usually three or four and many times only two.

Several features of system formation should be analyzed because of the changes in the intersiphonal patterns which are involved. Actually the existence of an intersiphonal band on each blastozooid greatly simplifies the task of observing the relationship of zooids to one another during the formation of systems. a. Is the form of a system (and accordingly of the intersiphonal pattern) identical whenever the same number of zooids forms one system? Is it identical even when different generations of zooids are compared? What factors affect the form of the intersiphonal pattern? b. What is the relationship between the number of zooids in a colony and the number of systems formed? Is there any inherent tendency for a colony to have a certain number of zooids per system, or is the number of zooids comprising a system purely a matter of chance? c. When an additional system is established, does it actually consist at first of a very small number of zooids?

When only two zooids constitute a system they typically occupy less than 90° of the space available around the cloaca and the form of the intersiphonal pattern is invariably the same, viz., the form of a letter V in which the angle between the intersiphonal bands is less than 90° . This is true whether the zooids involved are second, third or fourth generation zooids. Beyond the fourth generation there are usually more than two zooids per system, although in some colonies only a single zooid is present. If three zooids constitute a system the form of the intersiphonal pattern is slightly different depending upon the generation of zooids involved. If the zooids are of the second generation they occupy only 90° of the space available around the cloaca and an angle of approximately 45° is formed between adjacent intersiphonal bands. If the three zooids are third or fourth generation zooids they occupy 180° of the available space around the cloaca and an angle of 90° is formed between intersiphonal bands. If the three zooids involved are fifth or sixth generation zooids they tend to occupy all of the available space around the cloaca and the angle between adjacent intersiphonal bands usually increases to 120° . When systems consist of four zooids they tend to occupy all available space around the cloaca and adjacent intersiphonal bands usually form an angle of 90° with one another, although if third generation zooids are involved, less than 360° of the space is filled and the angle is correspondingly less than 90° . If there are five to ten zooids per system they are arranged radially around the cloaca filling all available space, with equal angles between adjacent intersiphonal bands regardless of the generation under consideration (Pl. I, Figs. 3 and 7). Whenever there are eleven zooids or more in a system the cloaca becomes oval shaped and the system itself is oval in outline instead of circular and the intersiphonal pattern becomes less of a perfect radial pattern (Pl. II, Fig. 9, the system on the right).

Several factors are important in controlling the form of the system. First, whenever possible a zooid tends to be in contact with another zooid on each side and throughout as much of its length as possible. Usually such

an arrangement is not possible until more than three zooids are present in a system, because three zooids or less are not usually enough to occupy all of the space around a common cloaca; thus the number of zooids available for system formation is a second factor determining the form of the system. The size of the zooids in the different generations is a third factor involved in the form of a system. Bancroft (1903) and Berrill (1941b and c) have both noted that the size of zooids increases progressively, with each generation. The larger the zooids involved, the fewer needed to occupy all the space available around the cloaca. If the above three factors affecting the form of systems are kept in mind it is possible to predict the form of any system in any colony providing the number of zooids and the generation of zooids is known. These factors exercise full control over the form of the system only when the system is isolated, i.e., only when there is a single system in a colony. When more than one system is present in the same colony the form of the systems is always modified due to crowding of zooids at the contact points between adjacent systems (Pl. I, Figs. 4 and 5).

Certain generalizations can be made concerning the relationship between the number of zooids in a colony and the number of systems formed. The maximum number of zooids which can constitute a single system is most commonly 14 or 15. Occasionally by the time 11 or 12 zooids are present the colony breaks up into two systems. If the colony consists of 16 to 22 zooids two systems are usually formed. If there are 23 to 30 zooids there are usually three systems. When the colony consists of 31 or 32 zooids four systems are usually present. When 52 to 55 zooids are present simultaneously they are most commonly distributed among six systems, and eight systems are usually found when as many as 61 or 62 zooids are present. Whenever the colony consists of more than one system of zooids the actual number of zooids within any one system seems to be determined primarily by chance. On the average there are eight zooids to a system, but there may be from four to fifteen in the colonies whose development has been followed. As far as can be determined there are no inherent differences between colonies in the number of zooids which constitute a system. This means that the number of intersiphonal bands of pigment cells which enter into the formation of any single intersiphonal pattern is purely a matter of chance. It would seem that if each new generation of zooids is larger than the previous one the number of zooids constituting a system should decrease with increased age of the colony. Such a tendency to decrease the number of zooids per system is not evident during the first seven generations of asexual reproduction. However, Bancroft (1903) has noted that in older colonies the number of zooids per system is reduced to three or four on the average.

The observations of Oka (1892), Pizon (1899b) and Herdman (1925) concerning the method of formation of additional systems seem open to question. According to Herdman (pp. 204-205), "When there is no longer room for all the ascidiozooids around the original cloaca, one or more may be pushed out into the surrounding test. Here they continue to form lateral buds and so found new systems." This would suggest that if more zooids are present than the maximum number which can be contained within one system, any extra zooids are crowded out singly from the old system and each of these

isolated zooids, by budding, then establishes a new system in the following generation. This implies that in order to form an additional system at least two generations of zooids must be involved: in the first generation one or more zooids become crowded out of the old system; in the second generation the buds developed from the zooids isolated in the previous generation actually establish the new system. It implies, moreover, that at the time an additional system is established the colony always consists of one large system plus the one or more very small newly established systems. Actually any such single zooids if crowded out of a system probably degenerate prematurely (see p. 96). Oka also emphasizes (p. 543) that "Ein neu gebildetes System enthält nur eine geringe Anzahl von Individuen, gewöhnlich deren drei oder vier, manchmal sogar nur zwei. Da aber bei jedem Auftreten einer neuen Generationen die Zahl der Individuen sich verdoppelt, so kommt es im Verlauf einiger Generationen zu einem typischen System von acht oder neun Individuen. . . ." However, elsewhere in his description Oka states (p. 542), "Nach dem Tod der Muttergeneration, welcher bald erfolgt, sieht man die jüngeren in ein System, häufiger aber noch in zwei gleich grosse oder ungleich grosse Systeme sich zusammenlagern." This latter statement of Oka alone accurately describes the formation of additional systems. Even before the death of the parent zooids the buds are roughly grouped according to the systems they will later form. And following the death of the parent zooids the newly formed zooids almost immediately become distributed into as many systems as are destined to form. There is no intermediate step during which all zooids first attempt to join around a single cloaca, followed by isolation of any extra zooids to establish smaller systems. Moreover, an additional system when it is first formed consists of a considerable number of zooids, Oka and Herdman to the contrary. In the ten colonies studied most extensively in this paper an additional system never contained fewer than four zooids when it was first established, and much more commonly it contained seven to nine zooids. Thus, when a colony contains sufficient zooids to form an additional system, the new system does not contain only the excess zooids which can no longer be accommodated in the old system, but rather the old system is replaced by two systems, each with approximately equal numbers of zooids.

Intersiphonal Bands as Taxonomic Characters

Any attempt to use slight differences in the appearance of intersiphonal bands as a basis for classifying *Botryllus* into species should be regarded with caution. Unless the taxonomist is fully aware of the progressive transformations which the intersiphonal bands undergo and of the time relationship between these transformations and the stages of asexual reproduction errors could be introduced by comparing the appearance of intersiphonal bands in colonies in entirely different stages of development. Moreover, even when colonies of the same stage of development are compared there will be differences in the number of intersiphonal bands in an intersiphonal pattern, as well as differences in the number of intersiphonal patterns in the colony, but such differences are due seemingly to factors which are not of taxonomic

importance. And even the absence of intersiphonal bands in some colonies may indicate nothing more than a single gene difference between these and colonies which exhibit intersiphonal bands.

SUMMARY

1. The most striking feature of many *Botryllus* colonies is the localization of special light-reflecting pigment cells between the oral and atrial siphons of each zooid. Collectively these pigment cells constitute an intersiphonal band.

2. Individual zooids of a *Botryllus* colony are distributed radially around a common cloaca as a system of zooids. Consequently all the intersiphonal bands within one system constitute a star-shaped pattern called an intersiphonal pattern.

3. Any *Botryllus* colony is characterized by a specific intersiphonal pattern for only a very brief period of its existence. Any given intersiphonal pattern is then totally destroyed within a few hours and is replaced by an entirely different pattern or group of patterns. The formation and subsequent destruction of intersiphonal bands is described in detail for the oozoid and first seven generations of blastozooids in living colonies established from isolated larvae.

4. The progressive remodeling of the intersiphonal patterns is intimately associated with the changes involved in asexual reproduction. An interval of four to five days occurs between the maximum development of one intersiphonal pattern and the maximum development of the next. During this brief period a typical series of changes occurs in the appearance of a colony. a. The zooids become highly sensitive and contractile and the intersiphonal pattern becomes very irregular. b. Zooids then undergo maximum and permanent contraction, the intersiphonal bands disintegrate and the pigment cells are released into the circulatory system and are carried passively by the blood stream. c. Simultaneously the new generation of zooids is growing and the new intersiphonal bands form in them. At first these are solid bands, but they tend to split lengthwise until they become double intersiphonal bands, especially in older generations of zooids. d. Reflecting pigment cells then disappear from the circulatory system.

5. There is no evidence that the pigment cells released by the break-down of the intersiphonal bands of one generation of zooids can be utilized in the formation of new bands in the next generation of zooids.

6. If the oozoid develops a *strong* intersiphonal band of pigment cells (i.e., a broad, almost solid band of reflecting cells between the two siphons) only *strong* intersiphonal bands appear on all later generations of zooids; if it develops a *weak* intersiphonal band (i.e., only a few reflecting cells near the dorsal midline with no obvious arrangement into a band) only *weak* bands appear on all later generations of zooids. This difference between *weak* and *strong* expression is constant and is probably genetically determined.

7. Intersiphonal patterns vary in different colonies even though they are in the same stage of development. These variations are discussed in terms of a. differences in the number of buds per zooid in different colonies, b. the premature atrophy of developing buds in some colonies, c. the possible functional persistence of parent zooids after their buds become functional, d. the fusion of adjacent colonies.

8. The form of an intersiphonal pattern is determined by several factors: a. Whenever possible zooids tend to come into contact with another zooid on each

side and throughout as much of their length as possible; b. the number of zooids available for system formation is a determining factor; c. the size of the individual zooids likewise affects the form of the pattern. If these three factors are kept in mind it is possible to predict the form of any intersiphonal pattern providing the number of zooids and the generation of zooids is known. When more than one system is present in the same colony the form of the intersiphonal pattern is always modified due to crowding of zooids at the contact points between adjacent systems.

9. When a colony contains sufficient zooids to form more than one system the new system does not contain only the few excess zooids which can no longer be accommodated by the old, but rather the old system is replaced by two systems, each with approximately equal numbers of zooids. As far as can be determined there are no inherent differences between colonies in the number of zooids which constitute a system.

10. The appearance of the intersiphonal bands or patterns should never be used as a characteristic for classifying *Botryllus* into species unless one is fully aware of the range of variation that occurs even in the same colony and of the factors responsible for such variations.

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